Effect of supplementation of vitamin C with yeast Saccharomyces cerevisiae on some blood parameters and body components in fingerlings common carp diets

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Abstract .
The present study was conducted to determine the effect of adding vitamin C with yeast S. cerevisiae on some blood traits. The experiment lasted for 60 days starting from 10/11/2023 until 10/1/2024. In a closed rotary culture system affiliated to the Fish Nutrition Laboratory, Department of Animal Production Technologies/Al-Musayyab Technical College/Al-Furat Al-Awsat Technical University. On fingerlings of common carp fish at a rate of 0.60±14.5 g. The results of the analysis showed significant differences between the experimental treatments and the control treatment T1, as treatment T2 excelled in RBC cell values, followed by treatment T3, while it was noted that with the increase in yeast treatment T4, RBC cell values decreased to 2.31 million / µl. The results of the white blood cell analysis showed that treatment T2 was significantly excelled ($P \leq 0.01$) on all other treatments at a rate of 26.21×103/ml3. It was noted that increasing the amount of yeast in treatments T3 and T4 led to a decrease in the number of white blood cells. As for the results of the hemoglobin HB analysis and PVC volume, the T3 and control treatments were significantly excelled ($P \leq 0.01$) to the other treatments. As for measuring the total protein level (TP), the results showed significant differences in favor of the three experimental treatments compared to control treatment. Treatment T4 recorded a significant improvement ($P \leq 0.05$), reaching 3.55 mg/dl. While no significant differences were recorded between treatments T2 and T3 compared to the control. While the results of blood sugar measurement showed that treatment T2 was significantly excelled ($P \leq 0.01$) at a rate of 45.10 mg/dL, compared to treatments T3 and T4, which in turn excelled on the control treatment. While the results of cholesterol analysis showed that treatment T3 was significantly excelled ($P \leq 0.01$) compared to the other treatments with an average of 141.0 mg/dL, while treatment T4 recorded a significant decrease in cholesterol levels at a rate of 125.0 mg/dL, which in turn led to the experimental treatments recording a significant decrease in triglyceride levels (glycerides) compared to the control treatment, which recorded high values of 194.50 mg/dL .

INTRODUCTION:

The expansion of fish farming practices for the species to be cultured requires a significant increase in the cost and quality of diets, which is one of the largest operating costs in aquaculture (Sultana et al., 2001). Over the past few decades, the aquaculture industry has emerged and has made significant contributions to animal protein worldwide, accounting for about half of the animal protein consumed annually (FAO, 2021). This development, in turn, has led to the release of large amounts of organic and inorganic materials, leading to the spread of many diseases, the degradation of the natural ecosystem, the collapse of fisheries, and the decline in biodiversity (Shafique et al.; 2021)). Which has greatly affected fish productivity and the general health status through the

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deterioration of water quality (Abdel-Latif et al.; 2022)), and as a result, these increases may lead to suppression of the immune system of farmed fish (Raza et al.; 2022). Therefore, it became necessary to use environmentally friendly food additives, such as probiotics, yeast supplements, vitamins to enhance growth, as well as antioxidants, and these additives have received greater attention in recent years (Naiel et al.; 2022). One of the most important is dry yeast S. cerevisiae, which is one of the most important types of probiotics most commonly used in its extracted form or in the form of a whole cell. Yeast has the ability to stimulate bile and regulate pH, in addition to being free of all types of plasmid-encoded antibiotic resistance genes and non-pathogenic. (Abu-Elala et al.; 2018) Yeast also plays an effective role in restoring the balance of microorganisms in the digestive tract flora to its natural form when fish are exposed to stress factors or disease (Bajagai et al.; 2016). Yeast protein is one of the most common single-cell proteins that is incorporated into aquatic feeds to effectively reduce the need for animal protein (Oliva-Teles et al.; 2001), due to its effective role in improving the health status of many fish (Korni et al.; 2021), including trout (Wache et al.; 2006), Nile tilapia (Abdel-Tawwab, 2012), sea bream (Dawood et al.; 2015), and common carp (Al-Jubawi and AL-humairi, 2024). Fish also need vitamins to survive because they act as enzymes and cofactors for metabolic processes within the body (NRC, 2011). Vitamins help aquatic organisms maintain optimal health and normal metabolic functions (Gasco et al., 2018), the most important of which is vitamin C or ascorbic acid, which plays a fundamental role in the physiological functions and normal growth of fish, as well as an effective role in blood parameters, biochemical characteristics and antioxidants inside the body (Cong-mei et al., 2022). Microscopically in the digestive tract when fish are exposed to stress factors or disease (Bajagai, et al.; 2016), and Saccharomyces yeasts were considered one of the most important probiotics that are used safely in fish nutrition when added to food components to control pathogens that support the health and growth standards of fish (Feldman, 2012 .(

S. cerevisiae is a beneficial microorganism and a source of single-cell protein due to its immune properties due to its glycoproteins and beta-glucan (Hamasalim, 2016). Vitamin C is absorbed in the anterior third of the digestive tract (Dabrowski, 1990). This was confirmed by the results of the study (Aboseaif, et al. (2022) that adding vitamin C to the diet of common carp fish significantly improved growth parameters and feed efficiency. Therefore, another unconventional approach was used in the current study by using and supporting the addition of one of the most important water-soluble vitamins to different levels of dry yeast S. cerevisieae in fish diets. Therefore, the current study aims to know the effect of adding vitamin C with different levels of yeast in improving the properties of diets by studying growth parameters, feed efficiency, body components and nutrients to fingerlings of common carp fish.

Materials and methods

The experiment was conducted in the Fish Nutrition Laboratory of the Department of Animal Production Technologies, Al-Musaib Technical College, for 60 days starting from 10/11/2023. 120 common carp fingerlings weighing 14.5 grams were used, distributed into eight treatments with 15 fish per replicate. The fish were fed 3% of their body weight.

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The environmental factors were somewhat stable during the experiment. Four diets were used in this experiment. And in the proportions mentioned in Table (1). The diets were formed for each of the treatments studied in the feeding experiment and according to the additives used in the experiment, as shown below:

•T1: Control diet (without any treatment.(
•T2: Control diet + 500 mg vitamin C only.
•T3: Control diet + 500 mg vitamin C + 2 g yeast/kg.
•T4: Control diet + 500 mg vitamin C + 3 g yeast/kg.

Table (1) shows the proportions of feed components added to vitamin C and different proportions of yeast S. cerevisiae in the manufacture of diets used in the experiment

No.	Feed materials (g/kg)	T1 control	T2	T3	T4
1	Fish powder	150	150	150	150
2	Soybean meal	350	350	350	350
3	yellow corn	100	100	100	100
4	Wheat flour	140	140	140	140
5	Sahala rice	100	100	100	100
6	Wheat bran	150	150	150	150
7	Premix	5	5	5	5
8	Vegetable oil	5	5	5	5
Total(g)	love	1000	1000	1000	1000
Additives	Vitamin C mg/kg	0	500 mg	500 mg	500 mg
	Bread yeast gm/kg	0	0	2gm	3gm
Chemical analysis % of feed components based on dry weight					significant
	T1	T2	T3	T4	
Moisture	7.50 ± 0.38a	6.95 ± 0.01a	6.02 ± 0.005 b	5.16 ± 0.03 c	**
Crude protein	29.61+0.32bc	29.06+0.25c	30.46+0.25ab	31.01+0.10a	*
Raw fat	4.60+0.09c	4.17+0.15c	5.29+0.14b	6.23+0.16a	**
Ashes	8.06+0.07b	8.58+0.11a	7.12+0.11c	6.30+0.05d	**
Carbohydrates	50.22+0.10c	50.68+0.05b	51.10+0.05a	51.29+0.08a	**

Blood image analyses on experimental fish:

Blood was drawn from different groups of fish at the end of the experiment directly through the heart (cardiac puncture), according to the method mentioned by Duman et al.,)2019), with 2 fish / replicate / treatment using 2 mm plastic syringes. Blood samples of 2 ml were drawn from each fish directly from the heart using a sterile syringe, the inner walls of which were moistened with EDTA anticoagulant, according to the method of Opiyo et al., (2019). The drawn blood samples were placed in the tube and moved directly gently after closing it with a rotational movement for 2-3 minutes, and the treatment

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and replicate number were fixed on it. The samples were kept refrigerated in a container required tests were performed on them, including the number of red blood cells (RBC), white blood cells (WBC), and hemoglobin (Hb), and the percentage of packed cell volume (PCV%) was measured, based on the methods mentioned by Coles, (1986.(

- Biochemical tests of blood serum samples:

The second part of the blood samples was placed in Eppendorf glass tubes without heparin to obtain blood serum. The tubes were left tilted at room temperature for half an hour, then placed in a centrifuge at 3000 rpm for ten minutes according to the method of (Yang and Chen, 2003). After that, the blood serum was withdrawn using a fine pipette tube, and stored in special tubes in the freezer at a temperature of -20 degrees Celsius. After that, the required tests were performed on the blood serum biochemically using a Spectrophotometer manufactured by the Japanese company Apel, using a special kit equipped by the German company Human. Serum biochemical analyses included estimation of (total protein, glucose and cholesterol.(

-The ready-made statistical program SAS was used to analyze the experimental data (SAS, 2001), and the significant differences between the averages were compared using Duncan's test (Duncan, 1955), at a probability level of ($P \leq 0.01$.(

Results and discussion

The results showed significant differences between the four treatments in all blood indicators under study, as the results of blood tests indicate the superiority of

-1 Water quality :

Throughout the duration of the experiment, the water quality was within the appropriate range for common carp (Cyprinus carpio L.), as the containing ice and transferred for the purpose of conducting the required analyses. The water temperature ranged between 22-27 degrees Celsius, the dissolved oxygen ranged between 4.5-6.5 mg/L, and the pH ranged between 7.81-8.43, while the salinity rate ranged between 0.63-0.54 parts per thousand. All of them were within the ideal limits for the living and growth of common carp.

-2 Number of red blood cells (RBC) and white blood cells (WBC :(

The results of measuring the number of red blood cells (RBC) in Table (2) show that there are significant differences ($P \leq 0.01$) in favor of the second treatment T2 compared to the rest of the experimental treatments, as the best values were recorded at 4.05 million / µl). While in treatment T4, when the yeast level increased, a decrease in the number of red blood cells (RBC) was observed, which was the lowest value. While the results of measuring the number of white blood cells (WBC) showed significant differences between the experimental treatments and the control treatment. Treatment T2 recorded a significant ($P \leq 0.01$) increase of 26.2 × 103 / ml3 compared to the other treatments. Also, treatment T4 shows a decrease in the number of white blood cells with an increase in the amount of yeast, which in turn recorded the lowest values at 24.25 × 103 / ml3.

-3Measuring hemoglobin (Hb) and packed cell volume (PCV): The results of the analysis of Hb and PCV% show no significant differences between treatment T3 and control treatment, while they recorded a significant difference ($P \leq 0.01$) on treatment T2 and the fourth T4. While it was noted that treatment T4 recorded low values in measuring hemoglobin Hb and blood cell volume (PCV%) with values that reached the lowest values of 3.40 g/dl and 12.30 %) when

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increasing the concentration of yeast in the formula of fingerlings diets of common carp.

Table (2) Effect of feeding levels of yeast S. cerevisiea on the blood picture in fingerlings of common carp Values represent (Mean±SD.(

treatments	RBC (million / µl)	WBC × 10^3 /ml^3	HB g/ dl	PCV (%)
T1 Cont.	± 0.04 3.16 b	± 0.10 24.70 c	± 0.056.25 a	± 0.10 21.4 a
T2 Vet.C(500mg/kg)	± 0.054.05 a	± 0.10 26.2 a	± 0.05 5.05 b	± 0.15 17.15 b
T3 Vet.C+2g S. cerevisiae /kg	± 0.053.15 b	± 0.15 25.25 b	± 0.056.25 a	±0.05 21.15 a
T4 Vet.C+3g S. cerevisiae /kg	2.31 ± 0.10 c	± 0.05 24.25 d	± 0.013.40 c	± 0.20 12.30 c
Significance level	**	**		**

*Vertical letters indicate a significant difference at the level of ($P \leq 0.01$.(

Probiotics play an important role in improving the health status of fish (Lapena, et al., 2020, as adding yeast S. cerevisiae to diets has a positive role in improving growth and health status (Tewary and Bidhan, 2011). The numbers of red and white blood cells can also be affected by internal or external factors such as pathogen infection and water pollutants, which in turn weakens the immune status of the organism (Grant, 2015). The results of the statistical analysis in the T2 treatment, which was added to it, showed an increase in the number of red and white blood cells, compared to other treatments .

The study Lunger (2006) indicated that the number of red blood cells in fish blood is affected by the type of nutrition, specifically the percentage and type of protein in the diet. This is what was observed in the second treatment, as dry yeast, when added in high proportions, produces CO_2 and alcohol, thus affecting the decrease in oxygen levels in the pond water (Legras et al., 2007). The results of this study are consistent with what was found by Ali et al. (2018), as no significant differences were observed in the numbers of red blood cells and white blood cells of Nile tilapia fed on diets containing commercial yeast. The results of the current study are consistent with the study of (Abdel Rahman et al. (2018) on Nile tilapia fish fed a diet supplemented with vitamin C for 28 days at a dose of 300 mg/kg, which gave better growth results and blood parameters compared to the control treatment. Also, the study of (Watson et al., 2008; Camevali et al., 2006) who mentioned that probiotics with vitamins play an important role in maintaining the integrity of cell membranes, which helps improve the health status of fish by regulating internal metabolic processes. Also, the results of the current study are consistent with the findings of Manoppo et al. (2015) when feeding Nile tilapia O. niloticus fish, as it stimulated immunity by increasing the number of WBCs, increasing the activity of phagocytic cells, and enhancing immunity. As for the analysis results for hemoglobin and packed blood cell volume, the results showed significant differences between the experimental treatments and the control treatment. These differences indicate the presence of effects the differences between vitamin C alone or with yeast on the one hand and compared with the control diet without any additions. This was confirmed by the study of Lunger (2006) in improving the measurement of hemoglobin and blood cell volume with the use of the ideal ratio of yeast and vitamins in the diets of cobia fish (Rachycentron canadum .(

This improvement may be attributed to the role of vitamins with yeast as well as the content of yeast on vitamins. The insignificant decrease recorded by the fourth treatment with the increase in the ratio of yeast with vitamin C is consistent with what was shown by the study of Al-Rifai (2016) on common carp fish when fed high levels of 10% yeast. However, the results of the study did not agree with what was found by Al-Faragi, (2014), who noticed an increase in the concentration of hemoglobin and the percentage of blood volume cells in diets supplemented with yeast only without adding vitamins. It is clear that the differences in blood parameters may be related to the species specifications and the formula of the feed. (Taati et al., 2011(

-4Measuring total protein and blood glucose. Total protein and Sugar Glucose The results of the analysis of total protein TP Table (3) indicate that treatment T4 was significantly excelled (3.55 g/dl) to the other experimental treatments, and no significant differences were recorded between them. As for the results of measuring blood sugar concentration, the experimental treatments recorded a significant superiority ($p \geq 0.01$) compared to the control treatment. While treatment T3 recorded the best values at a rate of (45.10 mg/dl) compared to treatments T2 and T4, which obtained lower values.

-5Examination of cholesterol and triglycerides in the blood .

The results of Table (3) show that significant differences were recorded between the three experimental treatments and the control treatment T1. It was noted that the third treatment T3 recorded the highest values in cholesterol levels in the blood of the experimental fish significantly ($p \geq 0.01$) and reached 141.0 mg/dl), while it was noted that treatment T4 recorded a significant decrease at a rate of 125.0 mg/dl with an increase in the level of yeast. As for the results of triglyceride analysis only, the results showed a significant decrease in values ($p \leq 0.05$) in the three experimental diets compared to the control treatment, which recorded high values at a rate of 194.50 mg/d.

Table (3) Total protein, glucose, cholesterol and triglycerides in the blood serum of fingerlings common carp.

treatments	Total protein level) g / dl(	glucose level (mg / dl)	Cholesterol level (mg / dl)	Triglyceride mg/dl
T1.Cont.	b ±0.05 3.25	c ±0.05 41.05	b ±0.5 134.50	a ±0.5 194.50

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T2: Vet.C(500mg/kg)	b ±0.05 3.15	b ±0.05 42.05	b ±0.10 131.10	b ±4.50 181.50
T3: Vet.C+2g S. cerevisiae /kg	b ±0.05 3.35	a ±0.10 45.10	a ±1.0 141.0	ab ±0.05 185.50
T4: Vet.C+3g S. cerevisiae /kg	a ± 0.05 3.55	b ±0.1042.10	c ±2.0 125.0	ab ± 0.50 186.50
significance level	*	**	**	*

* Vertical different letters indicate a significant difference.

Total protein plays an important role in maintaining the natural balance of the fish body and can transport many essential nutrients to the body through their association with it (Huang et al., 2008). The ability of vitamin C with yeast to stimulate protein synthesis in muscle tissue when feeding common carp fish may also explain faster and increase their weight compared to control diets (Faramarzi, 2012). It also represents the state of balance between the processes of building and demolition of body proteins, and the increase that occurred in the blood serum of the experimental fish under study indicates an increase in protein synthesis in the body and a decrease in demolition processes (Singh et al., 2017.(

Glucose is the main source of energy for most vertebrates, and it is a good indicator of the health status of the organism (Jana et al., 2016). The results show an increase in the level of glucose in the treatments added to yeast. This may be attributed to the content of the yeast wall on carbohydrate compounds (Munir et al., 2018). This was confirmed by the study of Abdel-Tawwab et al. (2008) in recording a significant increase in the level of glucose in Sarotherodon galilaeus fish in diets to which yeast was added compared to the control diet that was free of it. Hemre et al. (2002) also indicated that glucose levels in the blood plasma of fish are variable for several reasons, primarily the type and age of the fish and their feeding system. In general, the lower levels of yeast and vitamin C in the T3 treatment contributed to improving the blood sugar level, considering that the behavior of fish is similar to the behavior of mammals towards the pathological injury caused by high levels of blood sugar (Hepher, 1988). The study of measuring the level of cholesterol CH and triglycerides TG is one of the most important indicators that provide a comprehensive description of the health status of fish, as well as the most important changes that occur to explain and understand the metabolic process in the liver (Zhou et al., 2005). The presence of complex carbohydrates, vitamins and fibers in yeast helped reduce cholesterol levels, as well as the effective role of vitamin C, which prevents the deposition of harmful cholesterol in the digestive tract and prevents absorption (Safoura et al., 2010). In the same picture, this result was reflected in the results of measuring the level of triglycerides, which decreased in the three experimental treatments compared to the control treatment .

Yildiz (2006) indicated that yeast contains Phytoestrogens or so-called cholesterol and triglyceride-lowering agents when added to diet components. Or perhaps the decrease in cholesterol may be attributed to an increase in the production and secretion of organic acids by beneficial bacteria, whose growth and reproduction increase due to the biological

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precursor, which leads to inhibiting the effectiveness of enzymes that reduce the formation of fatty acids (Chen et al., 2014). Also, their levels decrease in the liver, which is considered the place of fat storage, to avoid fish being infected with what is called fatty liver syndrome (Hosseinifard et al., 2013). 6-Body components: Table (4) shows the chemical composition of fingerlings of common carp fish, indicating significant differences (P≤0.01) in the body content of various elements. The results show a significant decrease in the moisture content in the third and fourth treatments with an increase in the concentration of yeast in the experimental diets. As for the protein and body fat percentages, they recorded a significant increase (P≤0.05) compared to the second and control treatments before and at the end of the experiment. As for the ash content, the results showed a highly significant decrease (P≤0.01). It was noted that the ash content decreased in fish fed on diets containing different levels of yeast compared to the third treatment as well as the control treatment.

Table (4) Chemical composition (%) of experimental fish containing different percentages of bread yeast and fortified with vitamin C.

treatments	Moisture	Protein percentage	Fat percentage	Ash percentage
T1 control	± 0.0373.92 a	± 0.0317.88 b	5.85 ± 0.07 b	± 0.13 2.34 a
T2 Vitamin C 500 mg	± 0.0573.74 a	± 0.0117.80 b	± 0.085.89 b	± 0.02 2.56 a
T3 yeast 2% + vitamin C	± 0.1772.78 B	± 0.4118.40 b	± 0.256.93 a	± 0.011.87 b
T4 yeast 3% + vitamin C	72.12 ± 0.005 c	± 0.34 19.47 a	± 0.306.53 ab	± 0.031.87 b
p-value	**	*	*	**

Different letters vertically indicate a significant difference.

This was confirmed by the study of Laxmi, et al. (2013) that the addition of yeast to the diets of young Macrobrachium rosenbergii fish showed a significant superiority in the percentages of protein and crude fat in the muscle tissues of these fish compared to the control diet. Also, the study of Bagheri, et al. (2008) indicated that the addition of Bacillus spp. to the diets of rainbow trout led to a significant increase in the protein content of the fish body when compared to the control diet.

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